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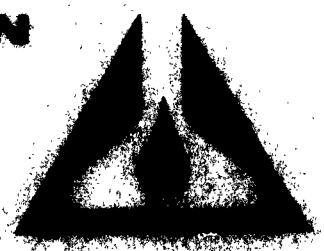

BIOCHEMICAL FUEL CELLS

Report No. 2

Contract No. DA 36-039-SC 90866

Task No. 3A-99-09-001-01

Second Quarterly Progress Report

1 October 1962 to 31 December 1962

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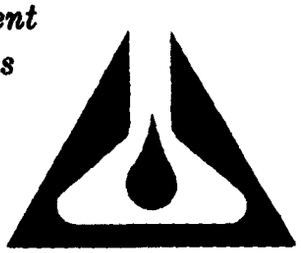

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The Object of Research: The Development of Electrochemical Power Generators Using Biochemical Reactions.

This report prepared by:

J. Brake

W. Momyer

Approved by:

H. Silverman, Project Leader

W. R. Scott, Division Manager

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1. PURPOSE

This report covers the effort under Contract No. DA-36-039-SC 90866 for the period 1 October to 31 December 1962. The purpose of this contract is to investigate means of utilizing biochemical reactions in electrochemical energy conversion devices. The first phase of the program is directed towards the investigation of pure biological systems operating on pure compounds, derivable from natural products, for the purpose of demonstrating feasibility, establishing the magnitude of energy involved, and developing promising approaches. Work has proceeded along two parallel tasks. Task I is concerned with the biochemical and electrochemical properties of selected biological systems while Task II is concerned with the preparation of biological coated electrodes.

Task I consists of the following three phases:

Phase I	Literature Survey.
Phase II	Cultural and Physiological Studies of Selected Systems.
Phase III	Electrochemical Studies.

Task II is divided into the following phases:

Phase I	Methods of Affecting Attachment of the Biological Phase to the Electrode.
Phase II	Study of the Electrochemical Parameters of the Electrode.

2. ABSTRACT

Investigation of the cultural and physiological behavior of the five biochemical systems previously selected for study was completed. The effects of pH and temperature on the activity of urease, cell-free extracts of E. coli and B. pasteurii are reported.

Current-potential data for all five biochemical systems (glucose-Cl. butyricum, glucose-cell-free extracts of E. coli, urea-urease, urea-B. pasteurii, leucine-L-amino acid oxidase) are included.

Studies were initiated on the electrochemical behavior of the biochemical reaction products, independent of the biological phase, for those systems where the products are isolable. Anodic current-potential curves of the urea hydrolysis product, ammonium carbonate, were obtained as a function of ammonium carbonate concentration and as a function of ionic strength of the anolyte.

A simple method of improving biological electrode performance by physically confining the biological phase at the surface of the electron carrier is discussed. Some preliminary current-potential data are reported on the urea-B. pasteurii system using this method.

3. PUBLICATIONS, LECTURES, REPORTS AND CONFERENCES

There were no conferences or publications during this report period.

A presentation on biochemical fuel cells was made to the Mojave Desert Section of the American Chemical Society by Dr. Silverman on January 7, 1963.

4. FACTUAL DATA

4.1 Introduction

The optimum utilization of biochemical reactions in an electrochemical energy generator depends ultimately on an understanding of both the biochemical and electrochemical reactions. Thus, the approach taken in this project is to study independently the biochemical and electrochemical kinetics of the systems selected for screening, and to then study the interaction of the biochemical and electrochemical reactions.

During the preceding report period the first phase of research on biochemical fuel cells under this contract was completed. This involved the selection of several organisms which appeared promising for application in portable power generators operating on naturally occurring fuels. Two of the organisms which were selected produce hydrogen: Clostridium butyricum, which metabolizes glucose, was chosen because it produces more hydrogen per mole of glucose than any other organism reported in the literature, and a cell-free extract of Escherichia coli which produces hydrogen from formic acid, was selected for study as a representative of cell-free systems in general. The other three systems which were chosen produce ammonia: Bacillus pasteurii is an especially active urea-hydrolyzing organism and will grow on waste materials; urease is an enzyme which is readily available and converts urea to NH_3 and CO_2; L-amino acid oxidase forms ammonia from several amino acids by means of an oxidation-reduction reaction.

These selected systems are being studied from two viewpoints. One is a biochemical, and involves the determination of the optimum conditions for the desired biological reaction involved. The second is electrochemical and involves the determination of the optimum conditions for the desired electrochemical reaction, e.g., oxidation of ammonia or hydrogen as the case may be. Finally, the best combination of the biochemical and electrochemical optima will be selected for use in a biochemical fuel cell.

During this report period, the biochemical studies were completed with physiological, cultural, and kinetic studies of B. pasteurii, urease, and cell-free extracts of E. coli. The electrochemical phase of this program was begun with polarization studies of the systems urea-urease, urea-B. pasteurii, glucose-Cl. butyricum, L-leucine-L-amino acid oxidase, and formate-E. coli (cell-free), and their products.

separate areas of the main compartment of a Conway microdiffusion dish (1). One ml of 1 N H_2SO_4 was placed in the center well and 5 ml of 5% K_2CO_3 in the outer well. When the dish was sealed, the sample was mixed with the KOH. After 18 hr the dish was opened, and the contents of the center well were analyzed for ammonia by Nessler's method (2). The pH optimum of this organism was determined by adding resting cell suspensions to 0.1 M buffers of phosphate and tris(tris-(hydroxymethyl)amino-methane) ranging in pH from 6.0 to 9.0 and containing 3% urea. After ten minutes the sample was placed in a Conway dish and analyzed for ammonia as described above.

4.2.1.3 Urease

The enzyme was purchased in partially purified form from a commercial source* who describe it as urease, 2XNF, B grade. Urease activity was determined by the procedure described above for assaying the ureolytic activity of B. pasteurii.

4.2.2 Polarization measurements

4.2.2.1 Three-necked cell assembly

Electrochemical studies of anodes in the absence of the biological phase were made in three-necked, glass half-cells which could be clamped together by means of a glass O-ring pipe fitting extending from each half-cell. An anion-exchange membrane (AR-111-A) was clamped between the two half-cells to prevent intermixing of the anolyte and catholyte. Each half-cell was fitted with a gas dispersion tube, a gas exhaust tube, and a saturated calomel electrode. Argon was sparged into the anolyte prior to the addition of the oxidizable material to remove the air from the anode compartment. Platinized-platinum foil electrodes (apparent area 10.75 cm^2) were utilized for both the anode and cathode. A photograph of the entire cell assembly is given in Figure 1.

The same platinized-platinum anode was utilized throughout this set of experiments to minimize surface area changes. This anode was cathodically cleaned in 1 N H_2SO_4

* Cal-Biochem, 3625 Medford St., Los Angeles 63, Calif.

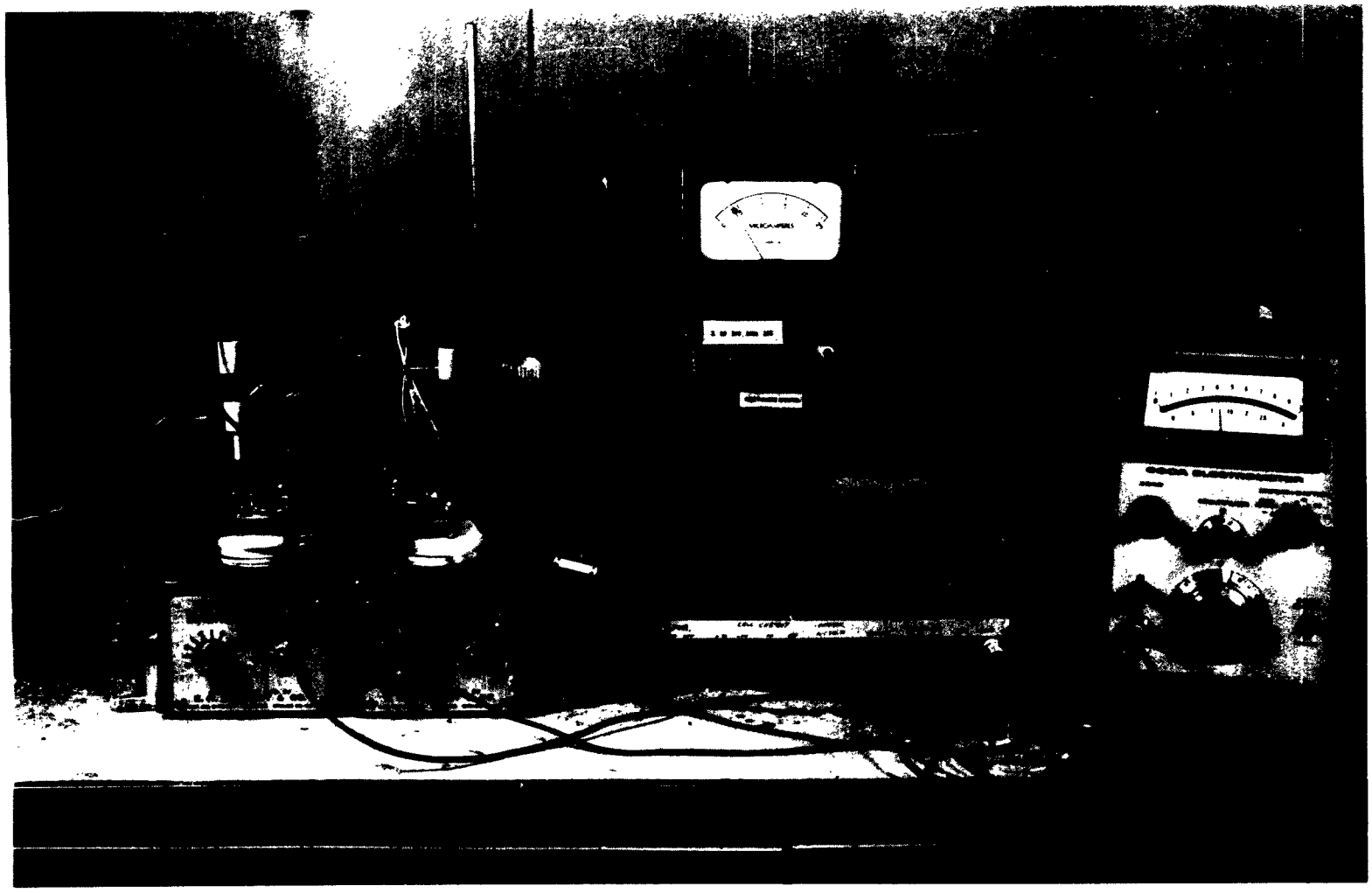

Figure 1 Photograph of Three-Necked-Cell Assembly

for 15 minutes at a current desnity of 10 mA/cm^2 prior to each experiment. An anodic current was then applied to this electrode to destroy the adsorbed hydrogen produced by the cathodic cleaning. Anodes treated in this manner gave reproducible potentials when immersed in ammonium carbonate solutions.

The anolytes tested during this report period were either ammonium carbonate dissolved in distilled water or in 1.5 molar sodium sulfate. These anolytes were buffered at a pH of 9.3 by the hydrolysis of the ammonium carbonate. Adequate stirring of the anolyte was accomplished by means of a mag-mix.

All the polarization data were obtained at ambient temperature. Polarization curves were measured both with increasing and decreasing current; the current level was set by means of a Magna electronic amperostat. A given current setting was maintained until a steady state potential was achieved on the anode. The anode potential was recorded with a Model MR Sargent recorder and the current was measured with a Simpson multi-range micro-ammeter. Internal cell resistance was measured at 1000 cps using an Industrial Instruments Conductivity Bridge.

An attempt was made to obtain polarization data with a Kordesch-Marko (3) bridge in order to compensate for any iR losses, but it was not possible to obtain steady state readings with this instrument. It was, therefore, necessary to obtain polarization data by conventional techniques. However, any error introduced in the anode potential measurements by not compensating for the ohmic voltage drop of the solution is small at the low currents involved in these experiments. The circuit of the Kordesch-Marko bridge is being rechecked to determine if it is at fault.

4.2.2.2 H cell assembly

Steady state polarization measurements on several systems were made in an H cell (E. H. Sargent & Co., S-29400). Platinized platinum electrodes with an apparent surface area of 9.3 cm^2 were used as the working electrodes and a saturated calomel electrode was employed as a reference electrode. The cell was deaerated with nitrogen. The measuring circuit contained a Simpson vacuum-tube voltmeter, a microammeter, a variable resistor, and a 6 volt battery. Polarization measurements were made by conventional techniques. The various electrolyte compositions which were tested in the H cell are

given in Table 1.

4.2.2.3 Compression type electrode

The compression type electrode was constructed as shown in Figure 2. The carbon anode was coated with a thick paste containing 500 mg of carbon black, 50 mg of platinum black, 100 mg of urea, and 50 mg of B. pasteurii (wet weight), mixed with sufficient tris buffer (0.1 M, pH 8.0) to make a paste. A cation-exchange membrane (Ionics, Nepton CR 61) was placed between the two electrodes. A glass tube, drawn to a capillary at one end and filled with saturated KCl in agar, was inserted into the anode paste and served as a salt bridge to a calomel reference electrode. The entire cell assembly was placed under pressure by turning a screw on the back plate, thus assuring good contact between the membrane and electrodes. Polarization measurements were made as described above.

TABLE 1. Electrolyte Composition for Polarization Measurements in the H Cell

System	Electrolyte	pH	Substrate
Glucose - Cl. butyricum	0.1 M sodium phosphate	6.8	34 mg/ml AC broth (Difco)
Urea-urease	0.1 M sodium phosphate 3 M potassium chloride	8.0	0.25 M urea
Leucine-L-amino acid oxidase	0.1 M L-leucine 0.01 M methylene blue	7.5	0.1 M L-leucine
Formate-Cell-free E. coli	0.1 M sodium phosphate	6.0	0.01 M potassium formate
Urea - B. pasteurii	1% nutrient broth, 0.5% yeast extract (Difco)	8.0	0.33 M urea

4.3 Cultural and physiological studies

4.3.1 Cell-free extracts of E. coli

Cell-free extracts were considered for study because they are intermediate in complexity between an enzyme and a bacterium. The cell-free extracts of E. coli, which produce hydrogen from formate, were selected for study because they are a particularly

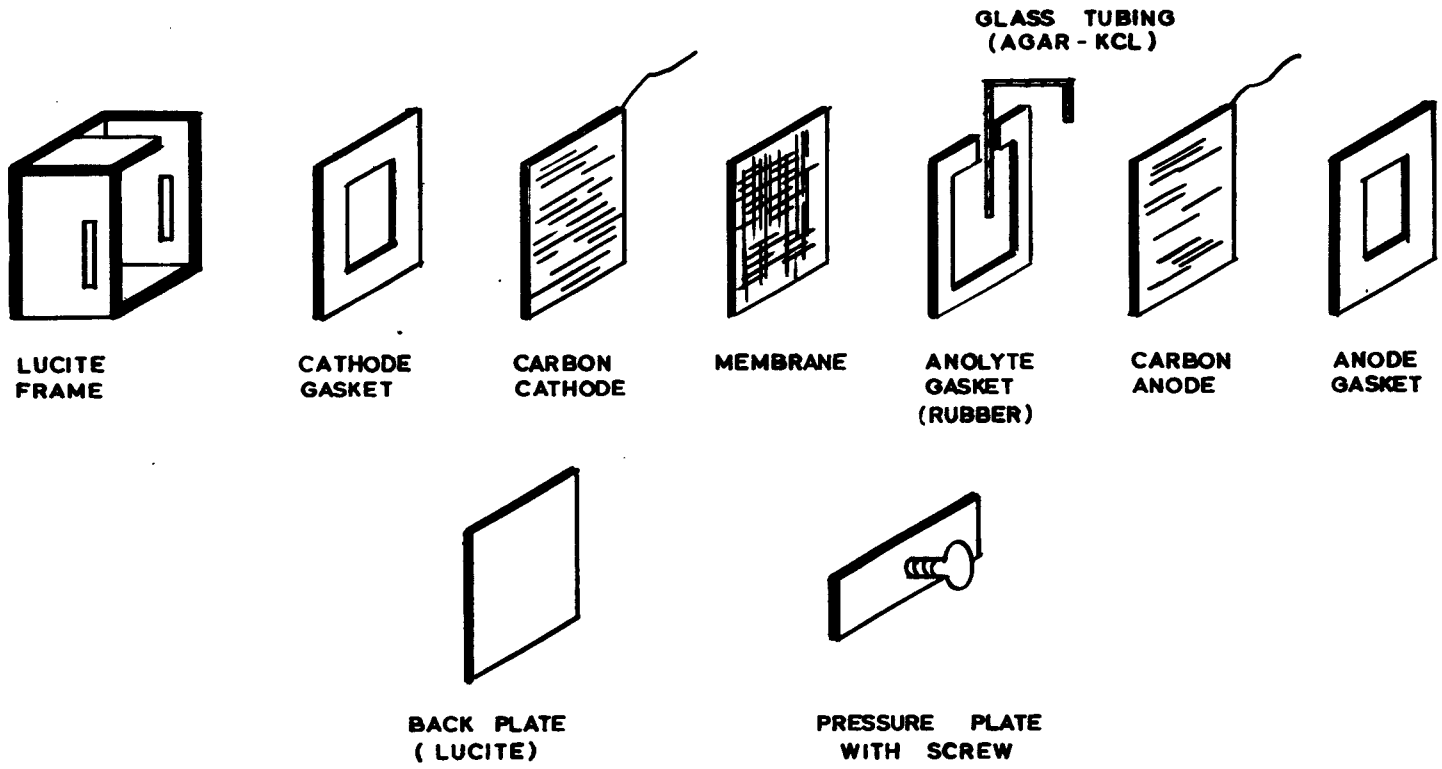

FIGURE 2. Schematic Diagram of the Compression Type Electrode in a Cell Assembly

well characterized system. The extracts were prepared by rupturing the cell walls and extracting the cell material with phosphate buffer (4). The extracts produce hydrogen from formate or pyruvate, but not from glucose, apparently having lost the capacity for glucose metabolism which the whole cells possess. A survey of the literature on cell-free extracts of E. coli indicated that it was necessary to grow the organism anaerobically in a medium enriched with amino acids to obtain the enzyme system which produces hydrogen from formate (5). The pH optimum for this system is 6.0 to 6.2, and little activity is found at pH 7.5 or higher (6).

The optimum temperature for hydrogen production by cell-free extracts of E. coli was determined by measuring hydrogen evolution in the Warburg apparatus at various temperatures (Fig. 3). From these experiments the optimum temperature was found to be 33°C in phosphate buffer at pH 6.0.

4.3.2 Studies of Bacillus pasteurii

Bacillus pasteurii was selected for study because it is an exceptionally active ureolytic organism, producing NH_3 and CO_2 from urea. It is capable of growing in urine, which makes it attractive for use in a fuel cell using human waste materials.

The strain which was studied was ATCC 6452. It was cultured in nutrient broth containing 3% urea. Ureolytic activity was measured by analysis for NH_3. The optimum pH was determined by testing its activity in buffers of several pH values (Fig. 4). The optimum pH for urea hydrolysis was found to be 7.0, and this differs from the optimum pH for growth, which was reported to be 8.8 (7). This is not surprising since the bacteria do not derive energy for growth from the urea hydrolysis reaction.

The organism was also grown in human urine. Good growth was obtained in shake cultures and sufficient ammonia was produced to change the pH from an initial value of 6.2 to a final value of 9.0.

4.3.3 Urease

Urease is present in jack bean meal and catalyzes the hydrolysis of urea according to the reaction:

$$CO(NH_2)_2 + H_2O = 2NH_3 + CO_2$$

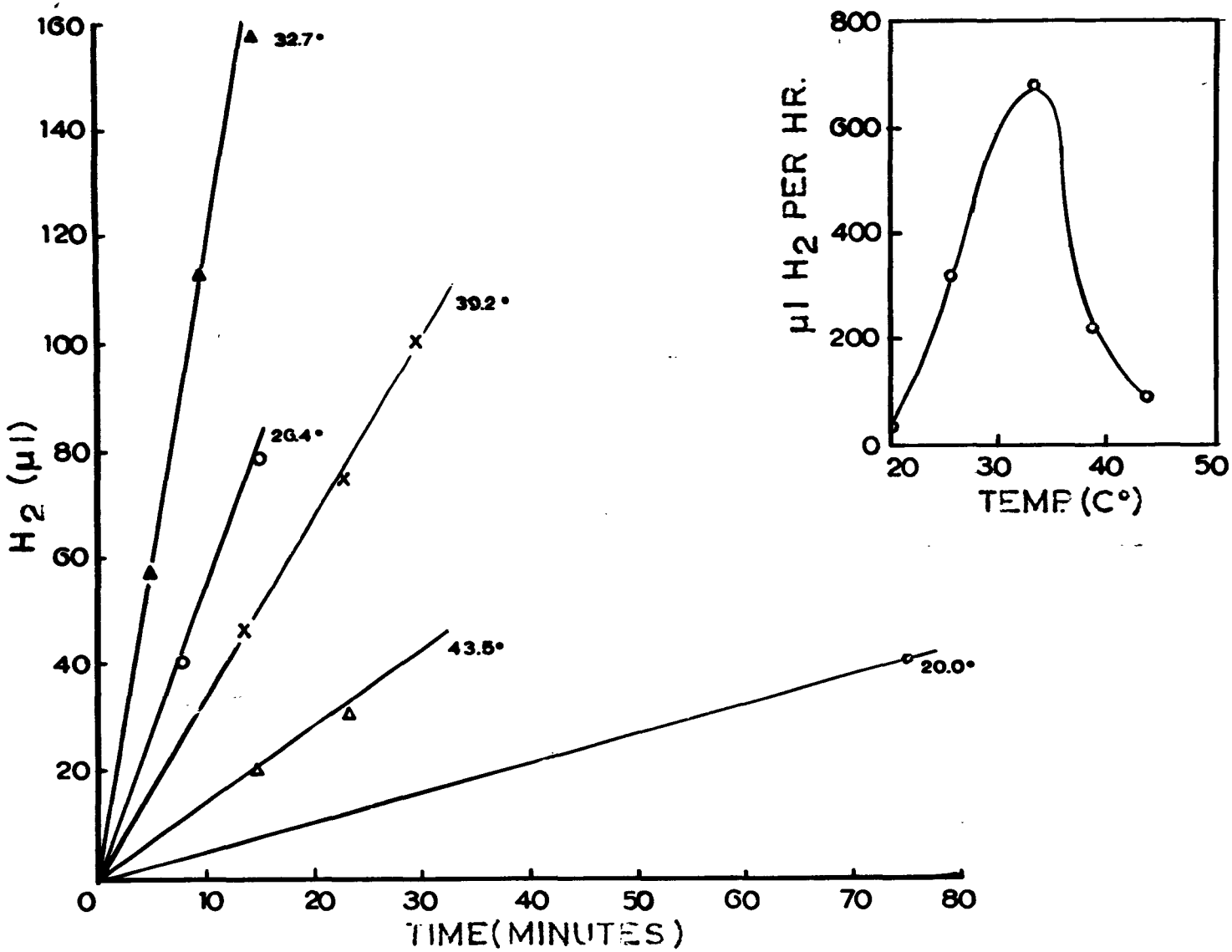

FIGURE 3. The Effect of Temperature on the Rate of Hydrogen Production by Cell-Free Extracts of E. coli Using Formate as the Substrate

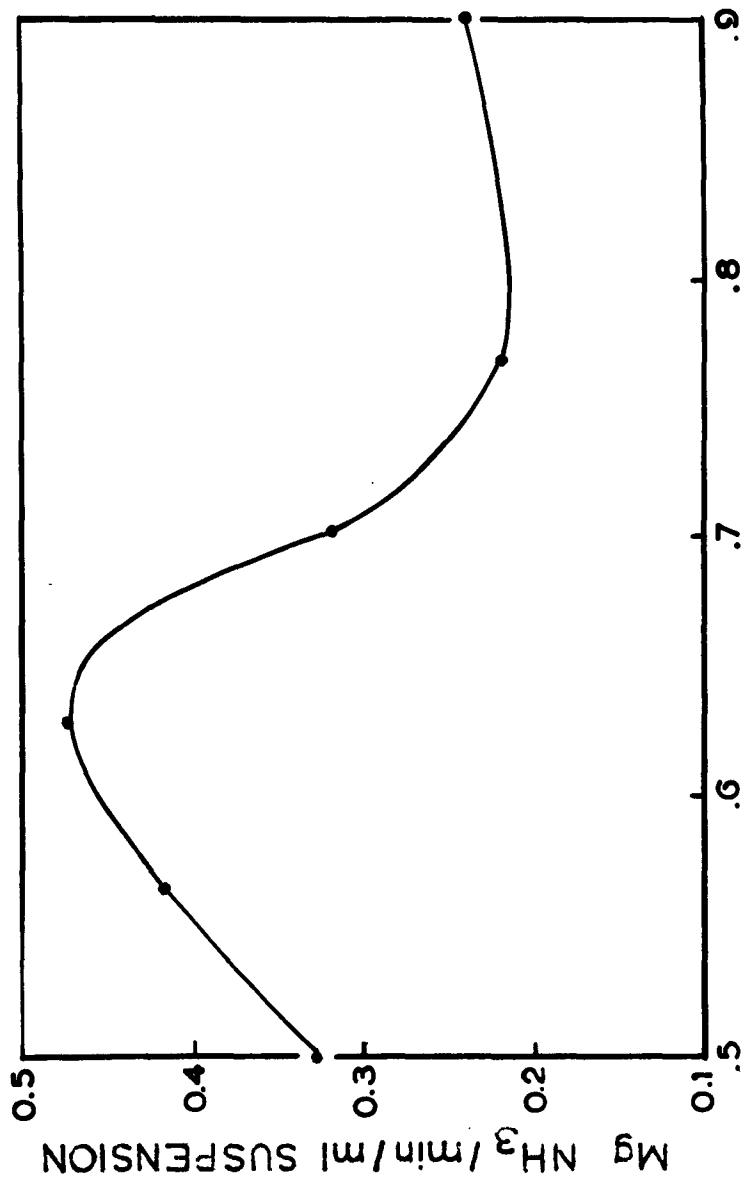

FIGURE 4. The Effect of pH on the Ureolytic-Activity of B. pasteurii

The enzyme has been studied extensively and the optimum conditions for ammonia production have been found to be: pH 8.0 in tris sulfate buffer (8), 0.2 M urea, and 45°C (9). The enzyme is inhibited by sodium and potassium ions (10).

Urease is available in crude form from Cal Biochem. While the preparation of the pure enzyme is not difficult, the amount of enzyme obtained is small (11); therefore, the crude preparation was used for these preliminary studies.

4.4 Electrochemical Studies

4.4.1 Electrochemical studies without the biological system

The biological hydrolysis of urea provides one of the most promising schemes for converting a substance readily available in the field into an electrochemically oxidizable species. This hydrolysis takes place efficiently and rapidly, producing two moles of ammonia and one mole of carbon dioxide for each mole of urea hydrolyzed. Assuming that nitrogen is the end product of the ammonia oxidation, $NH_3 \longrightarrow 1/2\ N_2 + 3H^+ + 3e^-$, six equivalents of charge are obtained per mole of urea hydrolyzed. The biological phase of this system (Bacillus pasteurii or urease) is relatively stable, active over a broad pH range (6-10), rather insensitive to high salt concentrations, and active up to a temperature of 47°C. Ammonia concentrations between one and two molar were easily obtained in the laboratory by the biological hydrolysis of urea, but difficulty was encountered in electrochemically oxidizing the ammonia under conditions compatible with the biological phase (particularly in the presence of the crude urease enzyme preparation). It was therefore deemed advisable to investigate the ammonia oxidation in more detail.

Since the hydrolysis of urea in aqueous solution produces ammonium carbonate, ammonia oxidation was studied using solutions prepared with reagent grade ammonium carbonate. Ammonium carbonate solutions are highly hydrolyzed and lose ammonia to form the bicarbonate (12):

$$2\ NH_4^+ + CO_3^= = NH_4^+ + HCO_3^- + NH_3$$

These solutions provide natural buffering action near a pH of 9.5. The following equilibrium is also established in ammonia solutions (13):

$$NH_3 + H_2O = NH_4^+ + OH^- \quad K = 1.77 \times 10^{-5}$$

Figure 5 shows the improved performance of a black platinum anode after addition of $(NH_4)_2CO_3$ to a 1.5 molar Na_2SO_4 solution. The anodic limiting current density increased from 100 μ A/cm^2 to 700 μA/cm^2 and the anode recovered more rapidly from severe polarization after this addition. Little change was noted in the internal cell resistance (decreased from 30 Ω to 28.5 Ω).

The variation in anode performance with increasing $(NH_4)_2CO_3$ concentration in a 1.5 molar Na_2SO_4 electrolyte is illustrated in Figure 6. It was noted that an $(NH_4)_2CO_3$ concentration of 0.25 molar resulted in the best anode performance in this electrolyte. Both higher and lower concentrations decreased the limiting current density.

TABLE 2. Effect of $(NH_4)_2CO_3$ Concentration on the Open-Circuit Potential and Limiting Current Density of a Black Platinum Anode Immersed in 1.5 Molar Na_2SO_4.

$(NH_4)_2CO_3$ Concentration	Limiting Current Density	OCP Anode vs SCE
0.05 molar	400 μ A/cm^2	-0.272 V
0.25 molar	700 μ A/cm^2	-0.262 V
0.50 molar	500 μ A/cm^2	-0.238 V

The internal cell resistance was approximately the same in all three experiments.

A study was made of the effect of ionic strength of the electrolyte on the performance of the aqueous ammonia anode. The effect on anode performance caused by the addition of Na_2SO_4 to a 0.5 molar $(NH_4)_2CO_3$ solution is shown in Figure 7. The limiting current density of this anode decreased from 1 mA/cm^2 to 700 μ A/cm^2 upon addition of the Na_2SO_4. The internal cell resistance also decreased appreciably

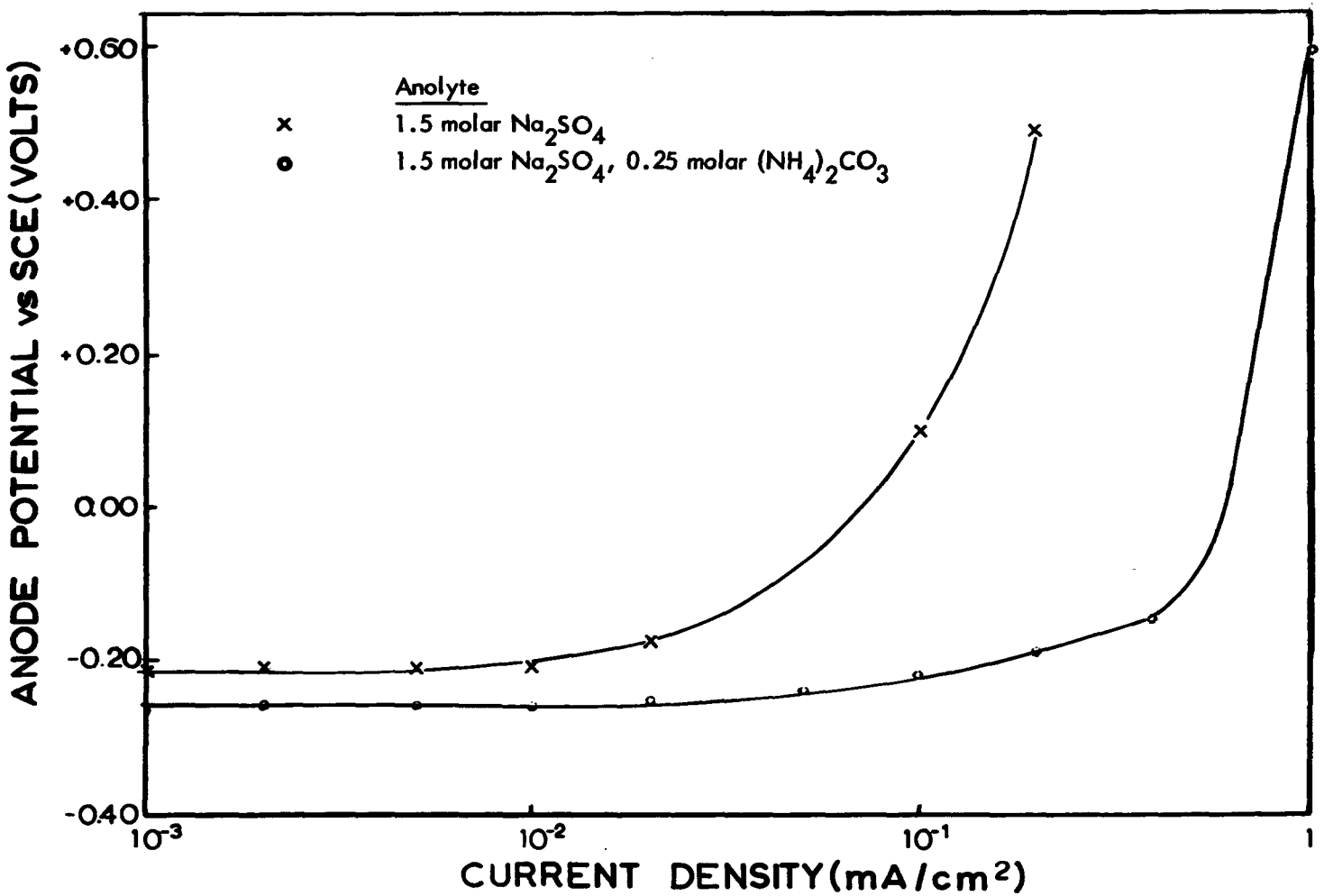

FIGURE 5. Effect of $(NH_4)_2CO_3$ Addition on the Performance of a Black Platinum Anode in 1.5 Molar Na_2SO_4 at pH 9.5

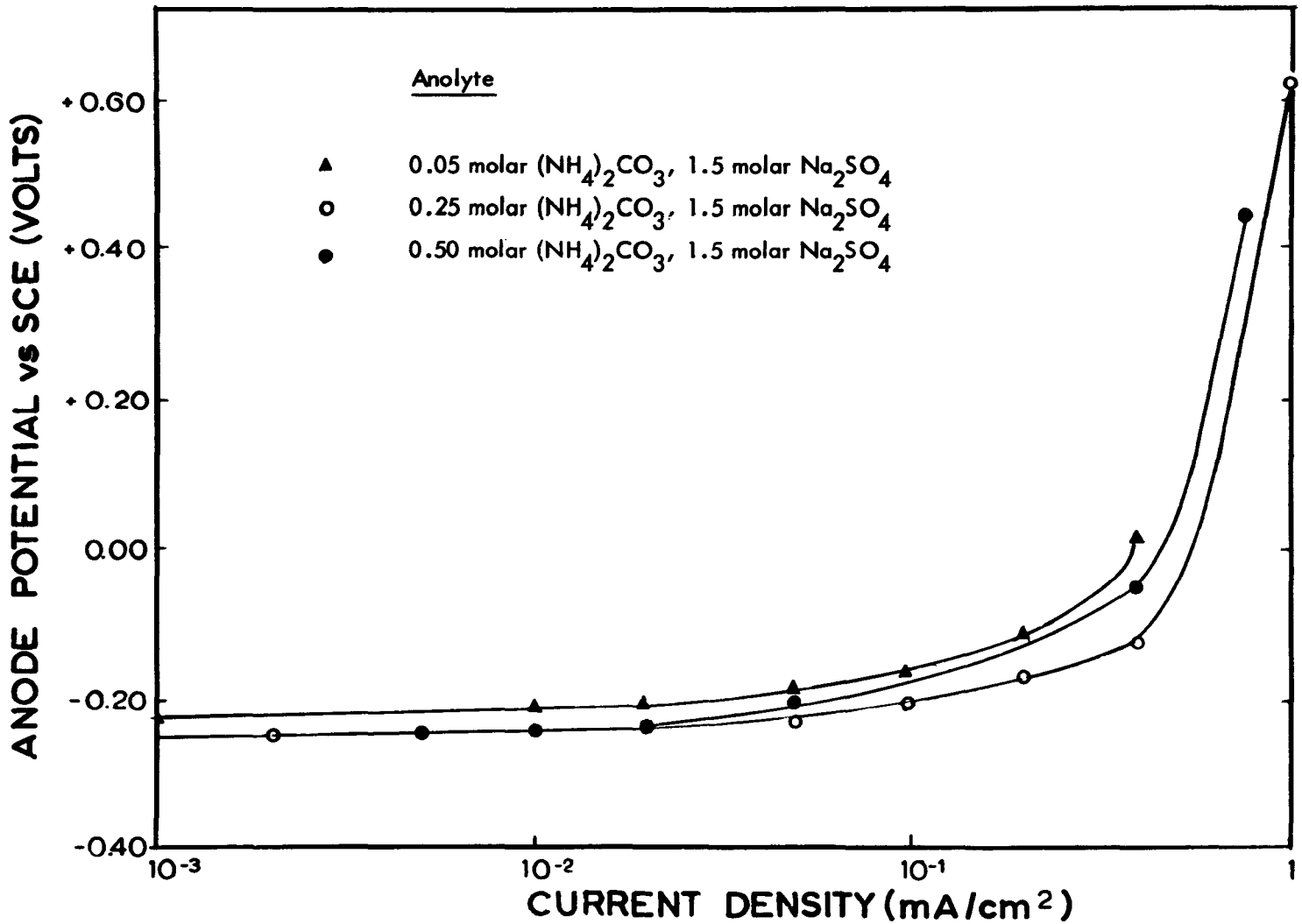

FIGURE 6. Effect of Increasing $(NH_4)_2CO_3$ Concentration on the Performance of a Black Platinum Anode in 1.5 Molar Na_2SO_4 at pH 9.5

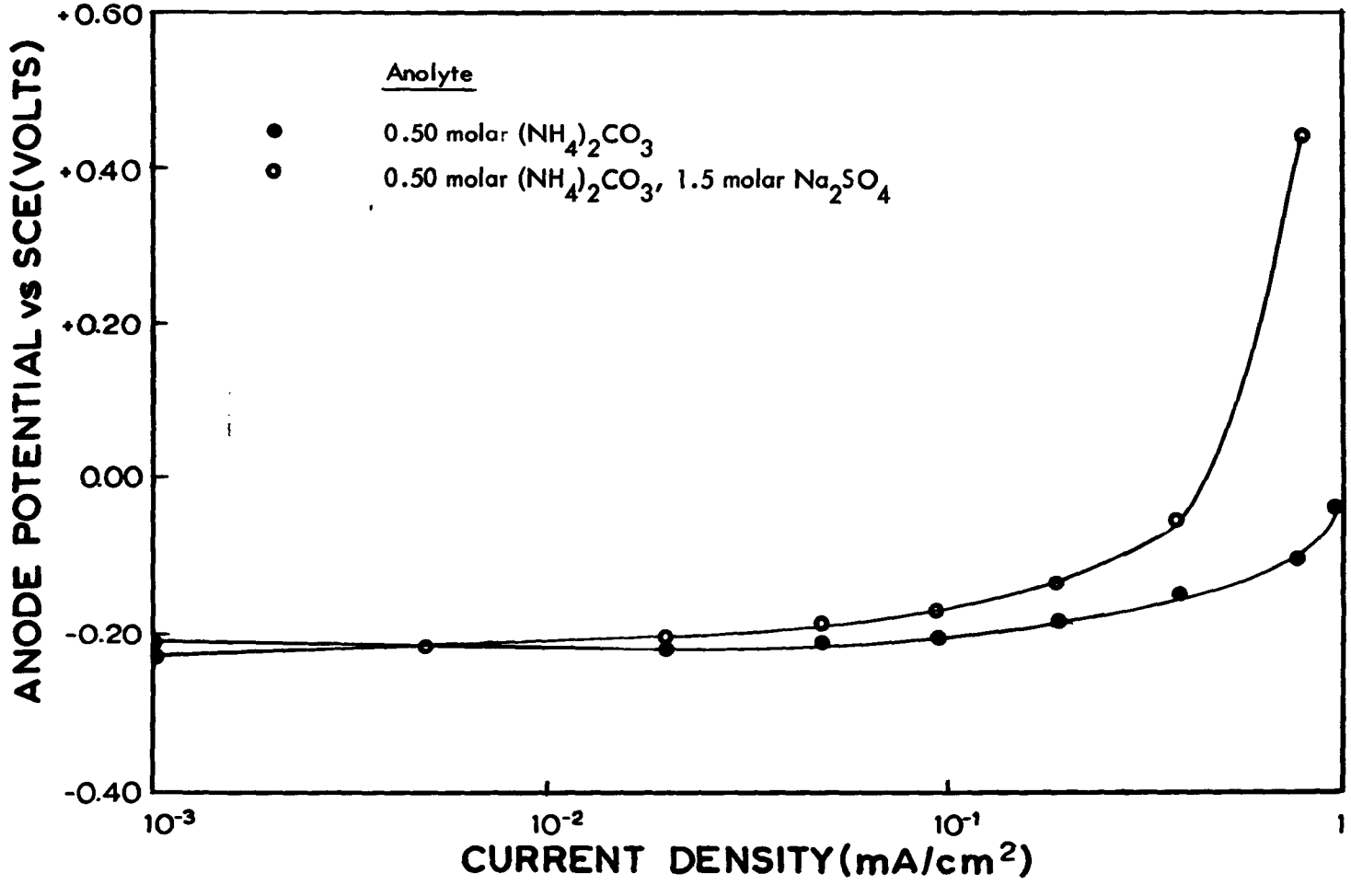

FIGURE 7. Effect of Na_2SO_4 Addition on the Performance of a Black Platinum Anode in 0.5 Molar $(NH_4)_2CO_3$ at pH 9.5

upon addition of this salt (40 Ω to 27 Ω).

Two rather unusual electrochemical characteristics of the aqueous ammonia anode were noted when the ionic strength of the $(NH_4)_2CO_3$ anolyte was increased with Na_2SO_4. First, a degradation in anode performance was noted upon increasing the ionic strength of the anolyte, and, second, the anodic limiting current density decreased upon increasing the $(NH_4)_2CO_3$ concentration above 0.25 molar. This behavior can be explained if ammonia rather than NH_4^+ is assumed to be the electroactive species. Thus, an increase in ionic strength of the anolyte shifts the ammonia equilibrium (13) and actually depletes the free ammonia present in the solution.

The effect of increasing $(NH_4)_2CO_3$ concentration on the polarization characteristics of a black platinum anode without extraneous salt in the anolyte is illustrated in Figure 8. In this case the anodic limiting current density was observed to increase slightly with increasing $(NH_4)_2CO_3$ concentration over the range investigated.

TABLE 3. Effect of $(NH_4)_2CO_3$ Concentration on the Open-Circuit Potential and Limiting Current Density of a Black Platinum Anode.

$(NH_4)_2CO_3$ Concentration	Internal Cell Resistance	Limiting Current Density	OCP Anode vs SCE
0.25 molar	70 Ω	700 $\mu A/cm^2$	-0.258 V
0.5 molar	40 Ω	1 mA/cm^2	-0.220 V
1.0 molar	32 Ω	1.3 mA/cm^2	-0.228 V

These data indicate improved performance of the aqueous ammonia anode without extraneous salt in the anolyte. The lower anolyte conductivity without this additional salt does not appear to offer a formidable problem at the levels of current density involved. An anodic limiting current density near 1 mA/cm^2 can be expected for an aqueous ammonia anode at ambient temperature with black platinum as the electrode catalyst. Improved anode performance is expected, however, with increasing temperatures

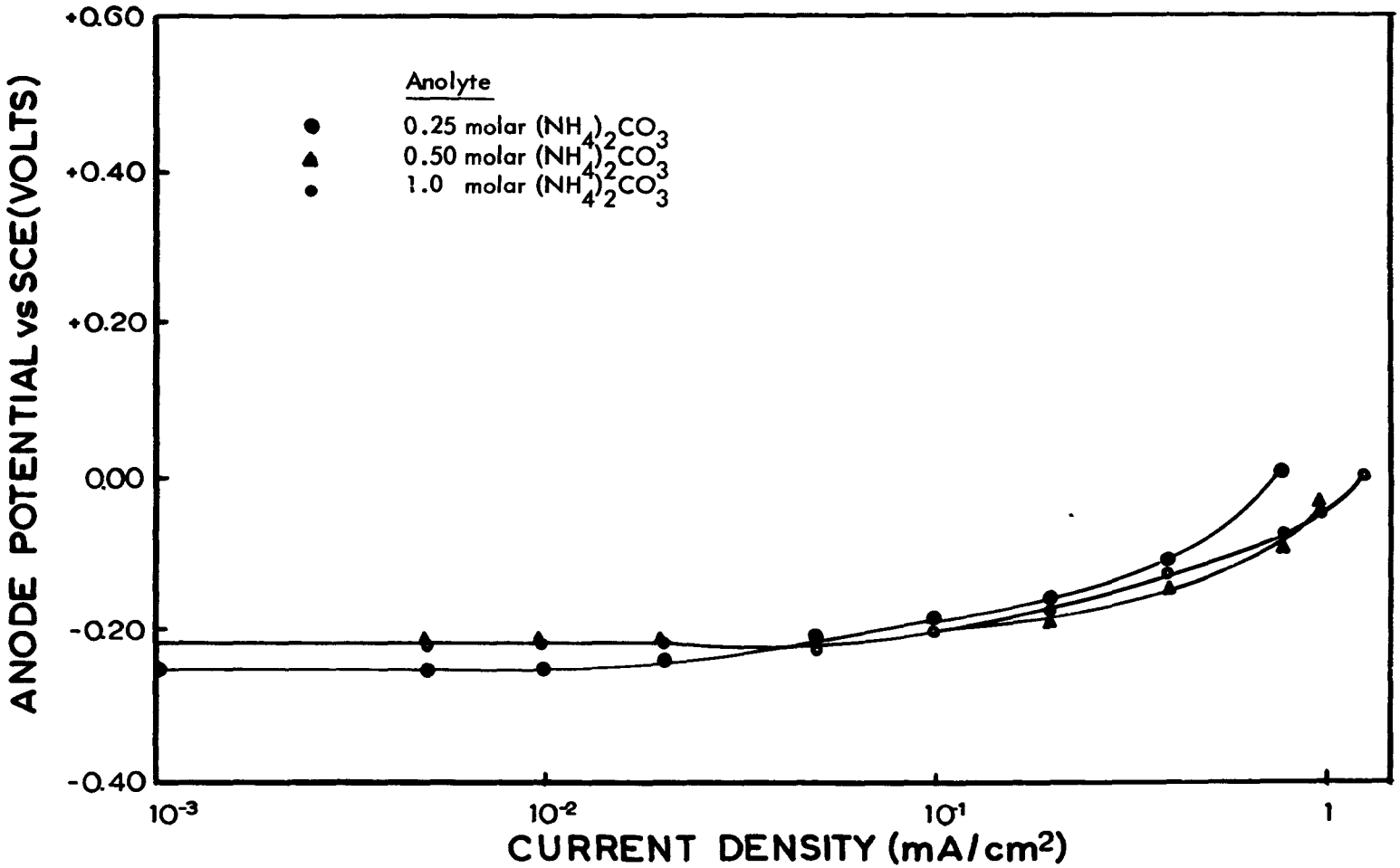

FIGURE 8. Effect of Increasing $(NH_4)_2CO_3$ Concentration on the Performance of a Black Platinum Anode at pH 9.5 without Extraneous Salt in the Anolyte

(up to a maximum determined by the properties of the biological system) and with black palladium as the electrode catalyst for the ammonia oxidation (14). Increasing the pH of the anolyte should also improve anode performance, but operation of a field battery at a pH other than that afforded by the natural buffering action of the urea hydrolysis products appears to be undesirable due to the difficulty in transporting the additional chemicals required.

4.4.2 Polarization measurements with biological systems

4.4.2.1 Clostridium butyricum

The addition of Cl. butyricum to AC broth (containing 0.01 M glucose and other nutrients to support the growth of this organism) at pH 6.8, which is the optimum pH for hydrogen production (15), changed the open circuit potential from 0.0 V to -0.63 V (vs SCE) and in general shifted the electrode potential about -0.6 V at any given current density. The limiting current density was also increased about 6 times from 0.07 ma/cm^2 to 0.4 ma/cm^2 (Figure 9). This was almost certainly due to oxidation of hydrogen, since this gas was produced copiously by the organism, and the measured open circuit potential (-0.63 volts vs SCE) corresponds to the normal standard potential of the hydrogen electrode adjusted to pH 7. This system remains especially attractive for use in biochemical fuel cells because at current-densities comparable to the other systems studied, it has the most anodic potential.

4.4.2.2 Cell-free extracts of Escherichia coli

When a similar experiment as described in Section 4.4.2.1 was performed with cell-free extracts of E. coli, using formate as the substrate, the electrochemical activity of the formate itself masked any activity due to the extracts (Figure 10). Since the extracts gave no improvement in electrochemical activity over that of formate itself, they will not receive further study.

4.4.2.3 Bacillus pasteurii

An enhanced electrochemical activity was obtained when B. pasteurii was added to a urea-containing solution at pH 8. However, it was found that the limiting current depended on the amount of bacteria in the cell (Table 4) so that with a bacterial protein concentration of 0.15 mg/ml, a limiting current of less than 0.01 ma/cm^2 was obtained

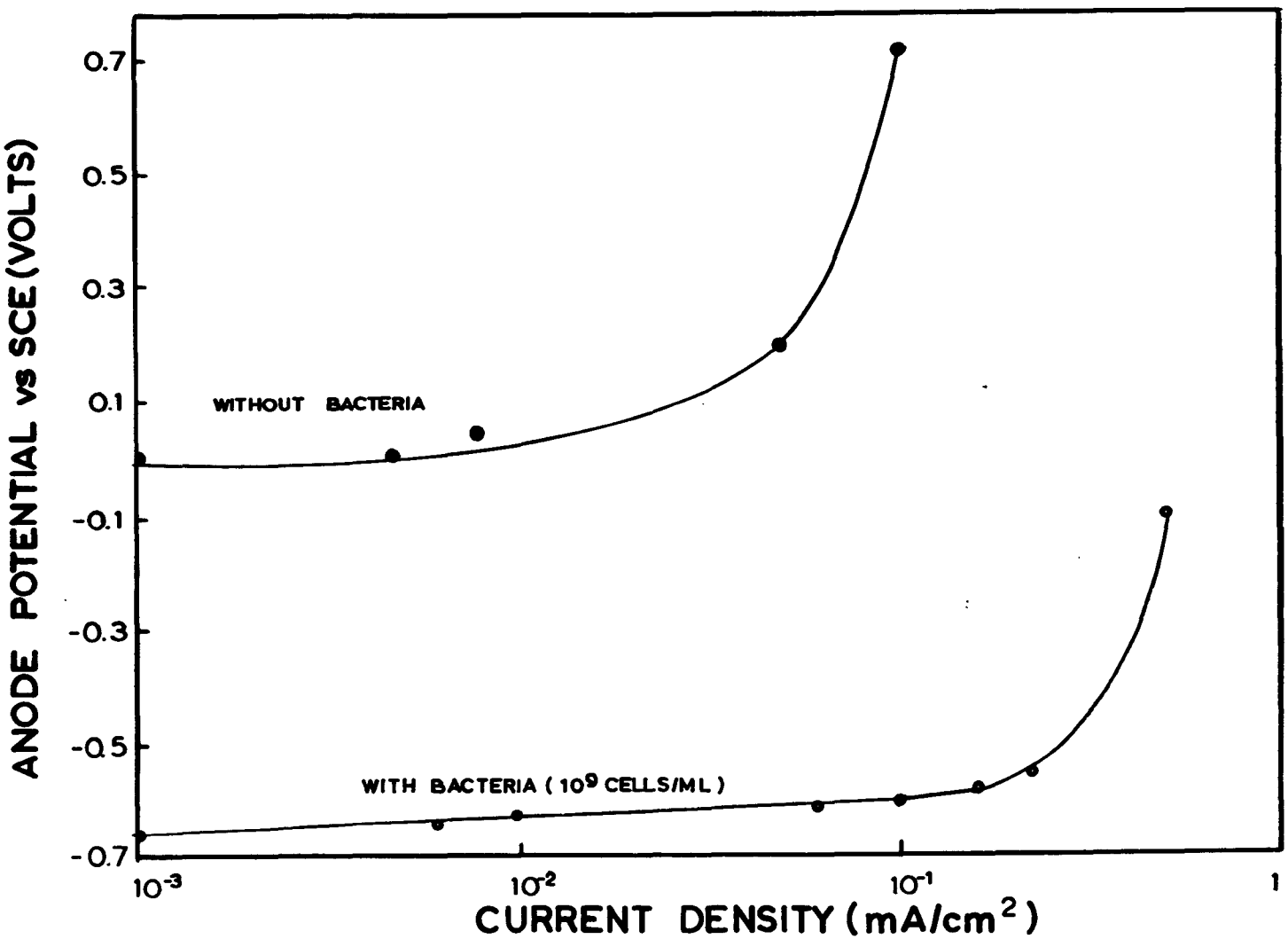

FIGURE 9. Current-Potential Curve of the Glucose-Cl. butyricum System in AC Broth at pH 6.8

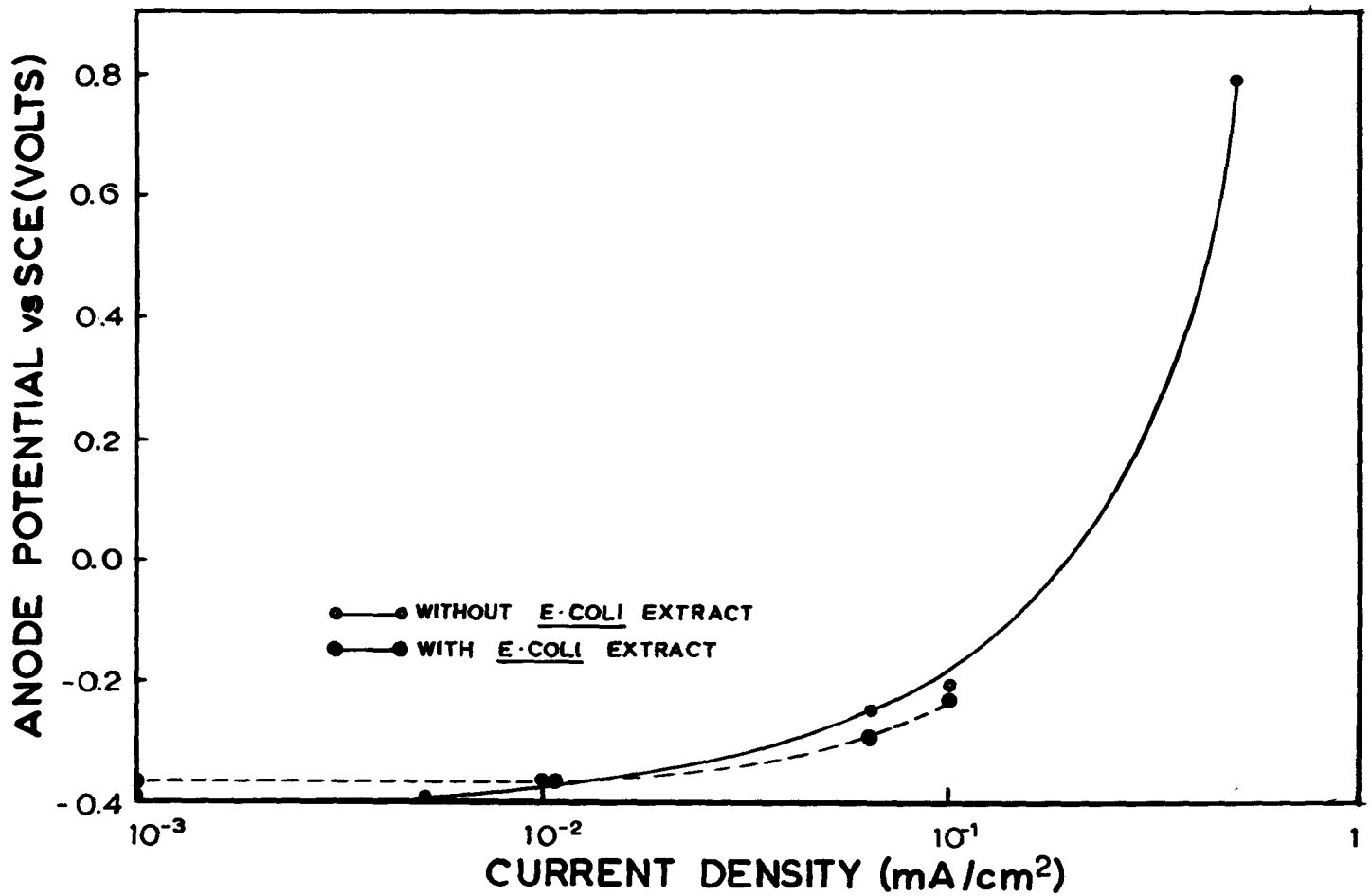

FIGURE 10. Current-Potential Curve of the Formate-E. coli (Cell-Free Extract) System at pH 6.0

but with 0.75 mg/ml the limiting current density increased to 0.2 ma/cm^2. However, a further increase in bacterial concentration did not increase the limiting current.

TABLE 4. The Effect of Bacterial Protein Concentration on the Limiting Current of the B. pasteurii Urea System

System	Open Circuit Potential (volts vs SCE)	Protein Concentration (mg/ml)	Limiting Current (ma/cm^2)
B. pasteurii - urea	-0.20	0.15	0.008
"	-0.24	0.30	0.04
"	-0.32	0.75	0.14
"	-0.26	2.4	0.08

4.4.2.4 Urease

The effect on electrode polarization of adding urea to a urease solution at pH 9.0 is shown in Figure 11. Some electrochemical activity was observed; the open circuit potential was -0.08 volts, and the limiting current density was 0.01 ma/cm^2. However, analysis of the electrolyte showed that the NH_3 content was 0.6 M, a concentration which gave a limiting current density of 0.1 to 0.2 ma/cm^2 in the experiments with B. pasteurii. It was also demonstrated that higher current densities could be obtained with concentrations of ammonium carbonate at this level by the experiments discussed in Section 4.4.1. A rational explanation of these results is that the crude urease is inhibiting the ammonia oxidation, and it follows that the low current densities observed with pressed carbon electrodes containing urease (16) are caused by the same inhibition. To determine if urease itself is inhibitory, further experiments with the purified enzyme will be performed.

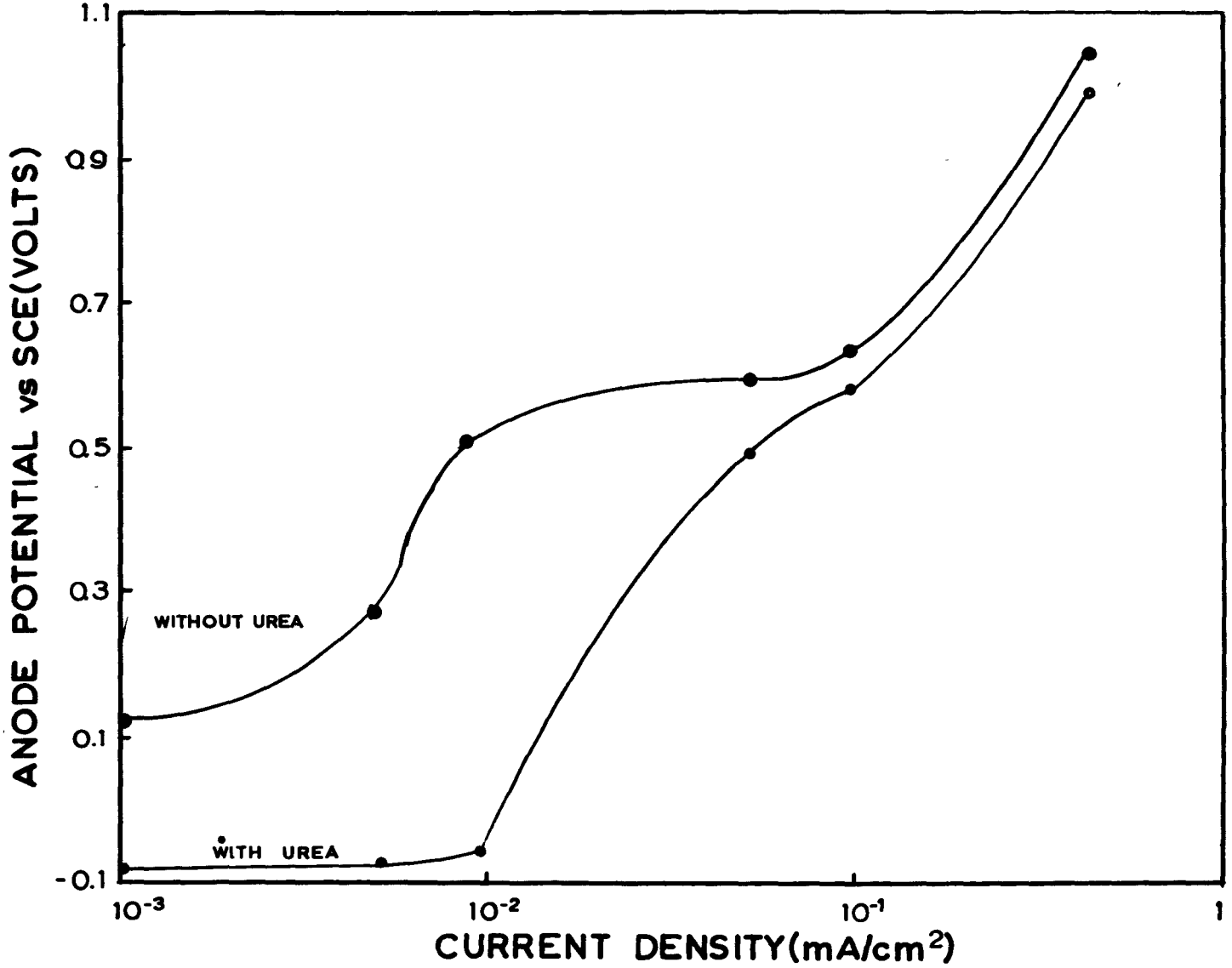

FIGURE 11. Current-Potential Curve for the Urea-Urease System at pH 9

4.4.3 L-Amino Acid Oxidase

L-amino acid oxidase requires an electron acceptor in order to promote the formation of NH_3 from amino acids. Oxygen is not suitable because it is competitive with the desired electrochemical oxidation, however, a number of organic dyes may be substituted for the oxygen. The dye chosen for the initial electrochemical studies was methylene blue, E (pH 7) = 0.011 volts vs the hydrogen electrode (17). With 0.01 M methylene blue solution and L-leucine as the substrate, an open circuit potential of -0.15 volts vs SCE at pH 7.0 and a limiting current of 0.08 ma/cm^2 (Figure 12) was found. Since there are two electroactive species produced by the reactions between the enzyme and the L-amino acid - reduced methylene blue and ammonia - it is difficult to predict what the equivalent weight of an amino acid will be. Two electrons are obtained from the electrochemical oxidation of the reduced methylene-blue and three from the oxidation of ammonia, so that a total of five faradays per mole of ammonia produced is possible. The faradaic efficiency of the system will determine the total number of ampere-hours available per unit weight. Since this enzyme acts only on some of the L-amino acids in a protein, the energy available from a hydrolyzed protein is not predictable.

4.5 Preparation of Biological Electrodes

Task II of this project is concerned with optimizing biological electrode performance. A close examination of the current-potential curves presented above reveals that in every case very little polarization is noted until the limiting current density is reached. The rate limiting step is as yet undefined. However, it is expected that if the biological agent could be retained at the surface of the electron carrier, much higher current densities would be obtained, due to reducing the diffusion path of the electroactive species. During this quarter the electrochemical characteristics of a biological coated electrode were investigated. A compression type electrode, described in Section 4.2, using membrane to retain the biological agent at the surface of the electron carrier (Figure 2) was prepared. The electron-carrier was coated with a thick paste of carbon black, platinum black, urea, and resting cells of B. pasteurii moistened with tris buffer, pH 8.0. Polarization of this

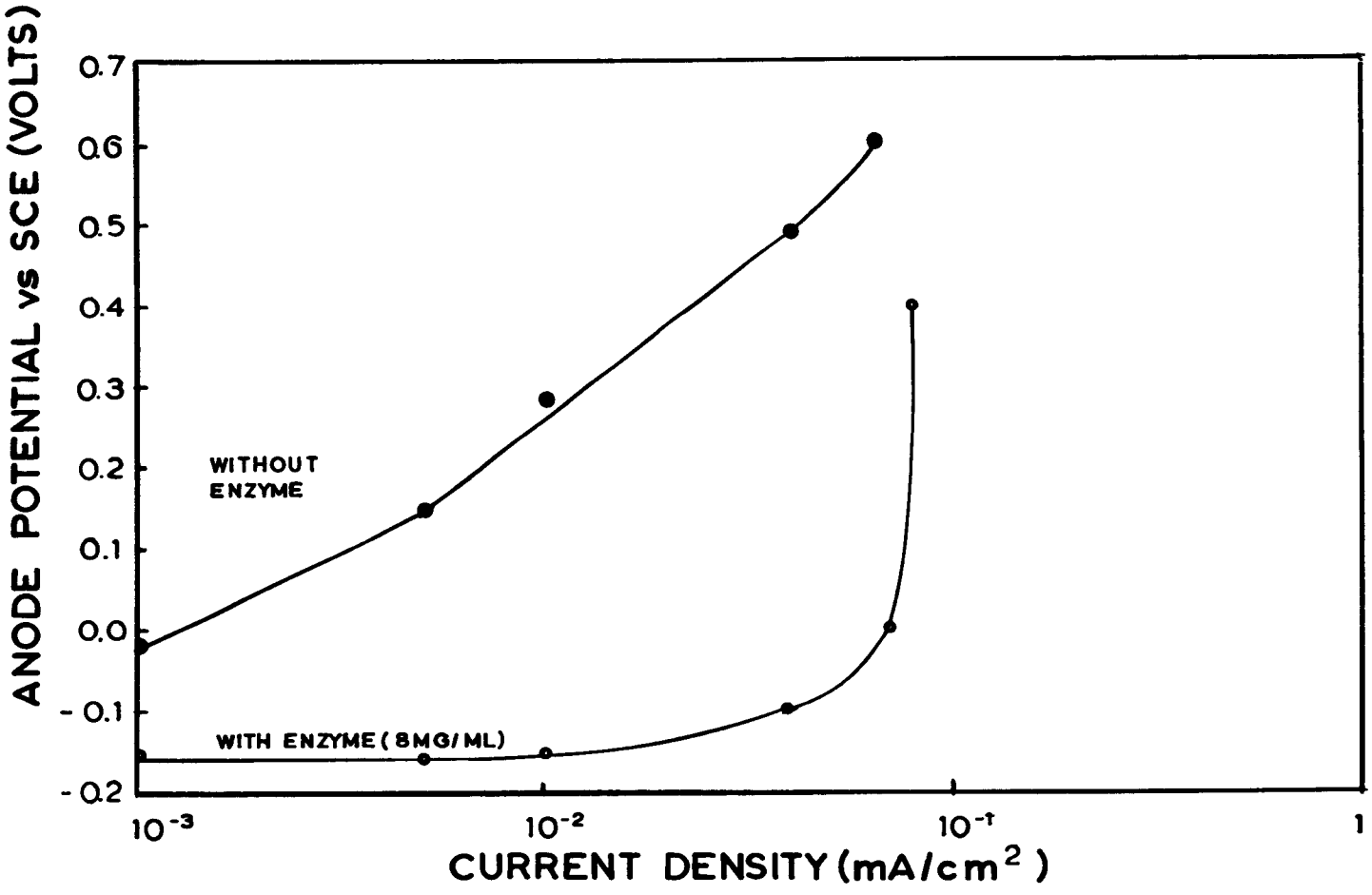

FIGURE 12. Current-Potential Curve for the Leucine-A-Amino Acid Oxidase System with Methylene Blue as the Mediator

system showed (Figure 13) an open circuit potential of -0.14 volt vs SCE and a limiting current density of 1.4 ma/cm^2. This represents a considerable increase in the limiting current density over that obtained in the H cell for the B. pasteurii system (0.2 ma/cm^2). Because of these results, it appears that the compression cell merits further study, and future effort will be directed toward testing other systems in this type of cell.

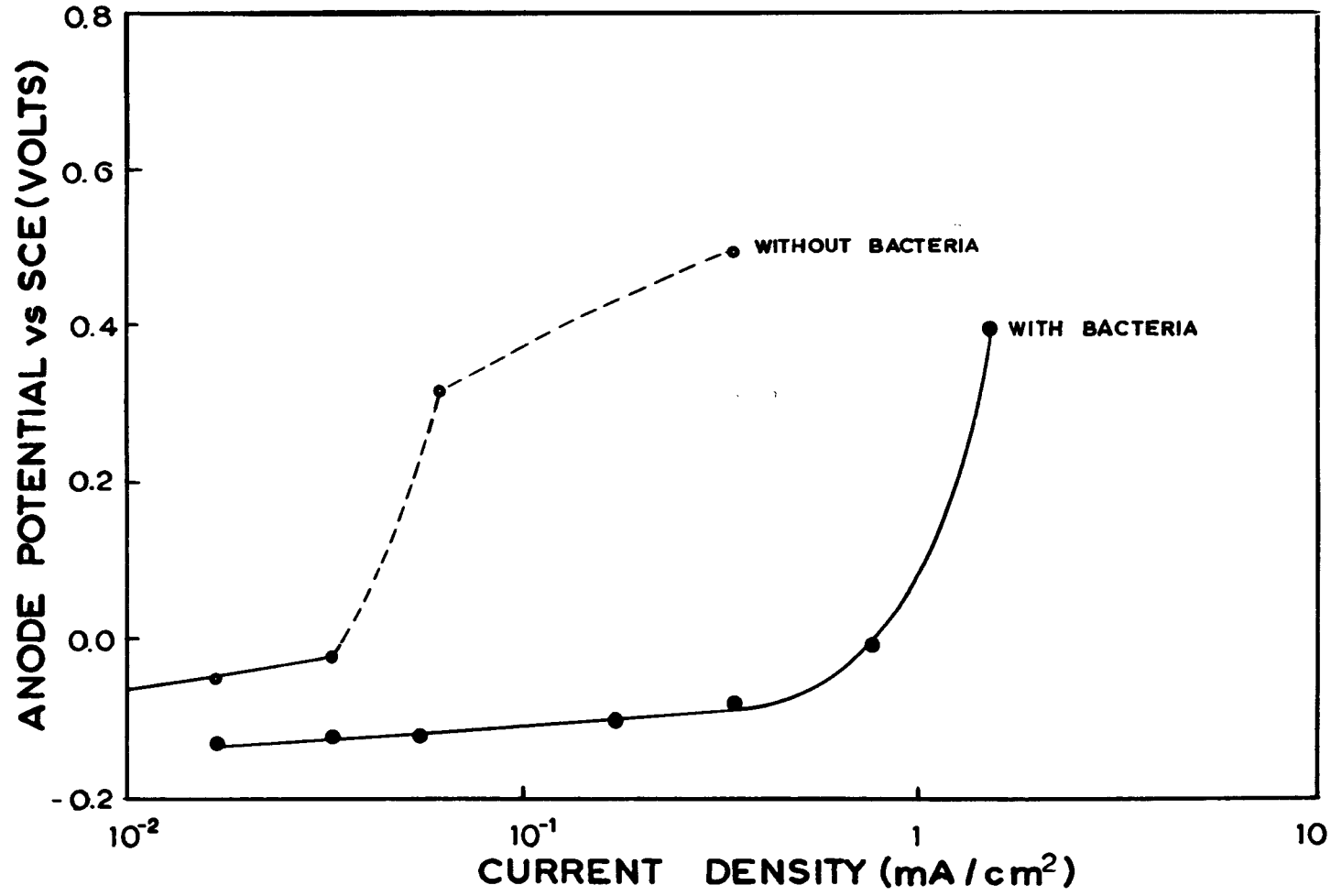

FIGURE 13. Current-Potential Curve of a Compression Type Electrode using the Urea - B. pasteurii System

5. CONCLUSIONS

5.1 Cultural and physiological studies of Escherichia coli, Bacillus pasteurii and urease yielded the following information:

1) The optimum pH for ureolytic activity of B. pasteurii is 7.0 to 7.5. This organism grows readily in urine.

2) The optimum pH for urease activity is 8.0 in tris buffer and the optimum temperature is about 45°C. Sodium and potassium ions inhibit the enzymic activity. The optimum substrate concentration is 0.2 M at pH 8.0 in tris buffer.

3) The optimum pH for hydrogen production from formate by cell-free extracts of E. coli is 6.0; the optimum temperature is 33°C.

5.2 Cell-free extracts of Escherichia coli are unsuitable for further study. The electrochemical activity of the substrate (formate) masks any effects due to hydrogen production by this system.

5.3 High concentrations (ca 20 mg/ml) of impure urease inhibit the oxidation of ammonia at a platinized platinum anode in ammonium carbonate solutions.

5.4 Thus far, limiting current densities on the order of 1 mA/cm^2 have been observed with the biological systems studied.

5.5 An improvement in the electrochemical performance of the aqueous ammonium carbonate anode was obtained by decreasing the ionic strength of the anolyte.

5.6 Improved performance was noted when a compression-type electrode of the urea-Bacillus pasteurii system was studied. The limiting current density observed in this cell was about ten times that observed in the H cell assembly.

6. PROGRAM FOR THE NEXT INTERVAL

6.1 Investigate the performance of the aqueous ammonium carbonate anode as a function of temperature.

6.2 Investigate the use of black palladium as the electrode catalyst for ammonia oxidation.

6.3 Quantitatively investigate the effect of impure urease addition on the performance of the aqueous ammonium carbonate anode.

6.4 Test the following biological electrode mixtures in the compression test cell:

1) Urease-urea-carbon-black platinum
2) B. pasteurii-urea-carbon-black palladium
3) Cl. butyricum-glucose-carbon-black platinum

6.5 Begin investigation of the glucose-glucose oxidase system as an example of a simple enzyme system involving no commonly accepted electrochemical fuel.

6.6 An analysis of the current-potential curves will be made and recommendations will be presented for determining the rate limiting step.

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8. IDENTIFICATION OF PERSONNEL

The distribution of hours of the key personnel assigned to this program for the second quarter is as follows:

	Hours
H. P. Silverman, Project Leader	79
J. Brake, Biochemist	384
W. Momyer, Electrochemist	206
S. Miranda, Technician	36
E. Nichols, Technician	114
Misc. Personnel	50

The biography of Mr. Miranda, recently assigned to this program is presented below.

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